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Study on preparation of probiotic yoghurt blended with fruits (Mango, Dragon and Apple)

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Abstract
The study aimed to create probiotic yogurt with mango, dragon fruit, and apple, using a 1% culture of *Lactobacillus casei* and *Lactobacillus plantarum*, along with a 1% yogurt culture, and replacing sugar with jaggery. Assessments covered nutritional composition, physicochemical characteristics, texture, sensory evaluation, storage dynamics, and packaging material influence. Results revealed nutritional values for the probiotic yogurt: Carbohydrate 19.12±0.17 g, protein 16.08±0.12 g, fat 35.55±0.25 g, and Energy Value 70.75±1.2 kcal. Standardized preparation methods and acceptability, gauged through physicochemical and sensory evaluations, compared different formulations (T_1 to T_4) with T_4 (79% yogurt, 21% fruit juice) receiving the highest sensory ratings. Storage analysis showed decreasing plate counts over 14 days, from 57 x 10^{12} to 9 x 10^8 for the control and 42 x 10^{12} to 21 x 10^8 for probiotic fruit yogurt. Yeast and mold counts remained negligible. Color variations were documented for the control (T_0) and selected sample (T_4).

Keywords: Probiotic, yoghurt, fruits, fortification, syneresis, viscosity

Introduction

The term "probiotic," derived from the Latin "for life," to characterize "substances secreted by one bacterium that promote the growth of another”. Probiotics are currently described as "live bacteria that, when supplied in suitable proportions, impart a health benefit on the host" as per the United Nations Food and Agriculture Organization and World Health Organization. Lactic acid bacteria and Bifidobacterium species are the most common probiotic microorganisms, both of which are “Generally Recognized as Safe” (GRAS) in the food business. The minimal number of probiotic cells (cfu/g) required for the function of probiotics' beneficial pharmacological (preventive or therapeutic) effects has been suggested to be expressed by the minimum of bio-value (MBV) index at the time of consumption. This index should be less than 10^7 cfu/g up to the date of minimal durability, according to the International Dairy Federation (IDF).

Yogurt is a fermented dairy product created through lactic acid fermentation using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria. Compared to plain milk, yogurt is considered more nutritious due to its higher concentrations of fat, lactose, protein, and minerals, which are also easier to digest and absorb. Yogurt is known to produce a microbial compound. The flavor, fragrance, and texture of yogurt are mostly attributed to *S. thermophilus*, which can also produce yogurt on its own. *L. bulgaricus'* function is to make acid. Typically, a 1:1 ratio of *S. thermophilus* and *L. bulgaricus* is inoculated simultaneously into yogurt milk (pH 6.6), however this ratio might fluctuate as the fermentation goes on.

Yogurt's global popularity stems from its numerous health benefits. The ideal fruit yogurt typically contains a fruit content ranging from 12% to 21%. Yoghurt enriched with seasonal fruits is especially enticing, as the addition of these fruits enhances its appeal and imparts a delightful taste. For a considerable duration, the global market primarily offered plain yogurt. However, in recent times, yogurt's popularity has seen a notable rise, mainly attributed to its enhancement through the addition of sugar and fruits. Presently, there exists a robust demand for fruit yogurt, especially among children, adolescents, and the elderly, who have a preference for the sweet and fruit-infused varieties.

The aim of the current study was to create probiotic fruit yogurt incorporating mango, dragon fruit, and apple, and to evaluate the physicochemical, nutritional, microbiological, and sensory attributes of this fruit yogurt.

Yoghurt is part of the diet in south eastern Europe and middle east for millennia and is now part of the dairy counters even in smallest grocery stores in many countries.

Materials and Methods

Buffalo milk, Fruits for pulp preparation, jaggery was collected from local market. Probiotic cultures such as *Lactobacillus plantarum*, *Lactobacillus casei*, and yoghurt culture were obtained from National chemical laboratory Dr. Homi Bhabha Road, Pune- 411008, India. Skim milk powder was procured from local market parbhani.

Maintenance of cultures

During the study period, the cultures were maintained in sterilized reconstituted skim milk (12 g Skimmed Milk Powder in 100 ml distilled water) tubes. The propagation of the cultures (at 37 °C) was done at weekly intervals to maintain their activity. The cultures were stored at 5 °C between the transfers.

Analysis

The probiotic plain and probiotic fruit yoghurts were analyzed for chemical, physical, microbiological and sensory properties.

Physicochemical properties of yoghurt during storage

The physicochemical parameters viz., TSS, pH, acidity, syneresis, viscosity, protein, fat, content of the samples was analyzed as per AOAC (2000) [1] methods. The pH was measured with a pH meter. Acidity was titrated by 0.1 N NaOH solution and expressed in terms of% lactic acid. The susceptibility of yogurt to syneresis was determined using drainage method.

Syneresis

Degree of syneresis expressed as proportion of free whey was measured. About 10 g of sample of yoghurt was placed on a filter paper resting on the top of a funnel. After 10 min of drainage the quantity of remained yoghurt was weighed and syneresis was calculated as follows:

$$\text{Free whey (g/100 g)} = \frac{\text{Weight of initial sample} - \text{Weight of sample after filtration}}{\text{Weight of initial sample}} \times 100$$

Viscosity

Viscosity measurements were conducted using a Brookfield DV-E Viscometer, employing Spindle No. 64 and a constant rotation speed of 6 rpm for all tested samples. The viscosity levels were continuously monitored during storage at a temperature of 4 °C, specifically after 1, 7, and 14 days. The recorded results were expressed in centipoise (cP) after subjecting the samples to shearing for 0.5 minutes. The viscosity of the yogurt underwent periodic analysis throughout the study duration.

Microbial analysis of developed product

The following media were selected as suitable for enumeration: MRS agar incubated at 42 °C for 24 h was applied for probiotic count and PDA agar was used for yeast and mould count. Microbiological count data are expressed as Colony Forming Units (CFU) per gram of yoghurt. Four dilutions were carried out to determine the number of bacteria during storage.

Statistical analysis

All the experiment were performed in triplicates and data were reported as mean ± Standard deviation. The Statistical Difference represented by superscripts were obtain using statistical software IBM (statistics v 26), one – way analysis of variance (ANOVA) at the probability level of $p<0.05$ and mean was compared by Duncan's multiple range test to check the homogeneity in variables while all the graphs are prepared by Microsoft office excel.

Standardization of plain yoghurt

The freshly activated yoghurt lactobacillus bulgaricus and streptococcus thermohillus was inoculated in buffalo milk (fat-6% SNF -9%) @ 1% and 2% level and incubated at 40 ± 2 °C for the preparation of yoghurt. Time taken for clean curdling was 5 hours. The acidity of yoghurt @ 1% and 2% level was 0.91 g % and 0.98 g % respectively. From the trial and sensory evaluation, it was found that 2% level of yoghurt inoculum used was found to be most acceptable. The acidity of plain yoghurt confirms with the FSSAI standards. The data obtained was statistically analyzed for mean ± standard error. From the statistical analysis, it was found that overall acceptability of yoghurt prepared with 2% inoculum level 1was found to be highly acceptable.

Standardize probiotic yoghurt blended with fruits (mango, dragon, and apple)

The standardized probiotic yoghurt was blended with mango, dragon, and apple fruit pulp @ 12%, 15%, 18% and 21% and 8g jaggery. The formulation was prepared by blending the mango, dragon, and apple fruit pulp with jaggery powder as presented below table 1. The proportion jaggery powder and probiotic culture in each sample was kept constant. To Standardized the formulation of product following variations were made.

Table 1: Standardize probiotic yoghurt blended with fruits (mango, dragon, and apple)

Material	T_0	T_1	T_2	T_3	T_4
Yogurt	100	88%	85	82	79
Mango Fruit	-	4	5	6	7
Drago Fruit	-	4	5	6	7
Apple Fruit	-	4	5	6	7
Jaggery	-	8	8	8	8
Starter Culture	2%	2%	2%	2%	2%

*Each value represents the average of three determinations

Flow Diagram for the formulation of probiotic fruit yoghurt

Buffalo Milk (6.5% fat, 9% SNF)
↓
Heated to 90 °C/10 min
↓
Cooling to 40 ±2 °C
↓
Inoculation with culture (yoghurt culture and Probiotic culture *Lactobacillus casei* and *Lactobacillus plantarum* (1:1) @ 2%)
↓
Incubation at (40±2 °C) for 5 hours
↓
Breaking the firm curd by blending for a min
↓
Addition of jaggery
↓
Addition of fruit pulp (mango/ dragon/apple fruit pulp @ 12, 15, 18 and 21%)
↓
Uniform mixing
↓
Filling
↓
Probiotic fruit Yoghurt (Refrigeration at 4±1 °C)

Flow 1: Diagram for the of probiotic fruit yoghurt

Table 2: Mean sensory characteristics on quality of probiotic fruit yoghurt

Sample	Sensory Attributes				
	Colour	Texture	Flavor	Taste	Overall Acceptability
Control	8 ± 0.05^{a}	8 ± 0.04^{a}	8 ± 0.06^{a}	$8\pm0.02a^{a}$	$8\pm0.00a^{a}$
T_1	8 ± 0.03^{a}	8 ± 0.05^{a}	8 ± 0.08^{a}	$8\pm0.04a^{a}$	$8\pm0.01a^{a}$
T_2	8 ± 0.03^{a}	8 ± 0.00^{a}	8 ± 0.00^{a}	$8\pm0.06a^{a}$	$8\pm0.00a^{a}$
T_3	9 ± 0.01^{a}	8 ± 0.12^{a}	9 ± 0.02^{b}	$8\pm0.00a^{a}$	$8.5\pm0.01b^{b}$
T_4	9 ± 0.02^{b}	9 ± 0.00^{b}	9 ± 0.01^{b}	$9\pm0.03b^{b}$	$9\pm0.03c^{c}$

*Each value represents the average of three determinations. Different subscripts in a column represent significant differences ($p<0.05$).

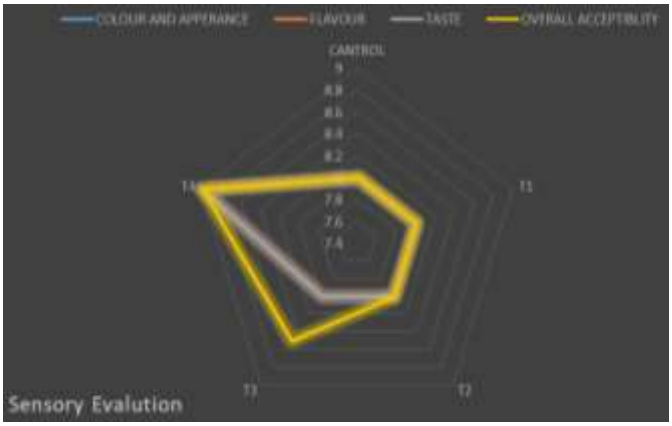


Fig 1: Sensory evaluation of probiotic yoghurt

In this context, probiotic yoghurt is prepared by introducing a combination of *Lactobacillus casei*, *Lactobacillus plantarum*, and yogurt culture, each at a 2% equal proportion. The variation lies in the concentration of fruit pulp used in the blending process.

Control yoghurt prepared from 0% blending of fruit pulp, T_1 probiotic yoghurt prepared from 12% blending of fruit pulp, T_2 probiotic yoghurt prepared from 15% blending of fruit pulp, T_3 probiotic yoghurt prepared from 18% blending of fruit pulp and T_4 probiotic yoghurt prepared from 21% blending of fruit pulp.

Colour plays a crucial role as an initial factor influencing the acceptability of food and serves as an indicator of the suitability of probiotic yogurt for consumption. It holds significant importance as a sensory attribute that directly impacts consumer acceptability of the product.

The findings in Table 2 indicate that the colour of probiotic yogurt remained consistent when the concentration of the starter culture was the same, with the only variation being in the concentration of fruit pulp used in blending. Sample T_4, which contained 21% fruit pulp, exhibited an acceptable colour. The maximum score for colour of probiotic yoghurt was obtained by sample T_4. The score of flavour of probiotic yoghurt from 8 to 9, among the probiotic yoghurt prepared from different level of blending. The probiotic yoghurt Sample T4. However the lower sample T_1 and T_2.

Taste in the health food is usually affected by characteristic changes occur during manufacturing. The maximum score for taste was in the sample T_4. While T_1, T_2 and control sample score is lower values. The taste sweetness was found to increase with increase concentration of fruit pulp blending.

The overall acceptability score is determined by considering various organoleptic quality factors, such as color, flavor, taste, texture, and more. It represents the collective perception and approval by the panel of evaluators. Among the samples tested, the highest overall acceptability score was observed in sample D, which was inoculated with a 2% equal proportion of *Lactobacillus casei*, *Lactobacillus plantarum* and yogurt culture, along with a 21% blending of fruit pulp. On the other hand, the lowest overall acceptability scores were recorded for the control sample, as well as T_1 and T_2.

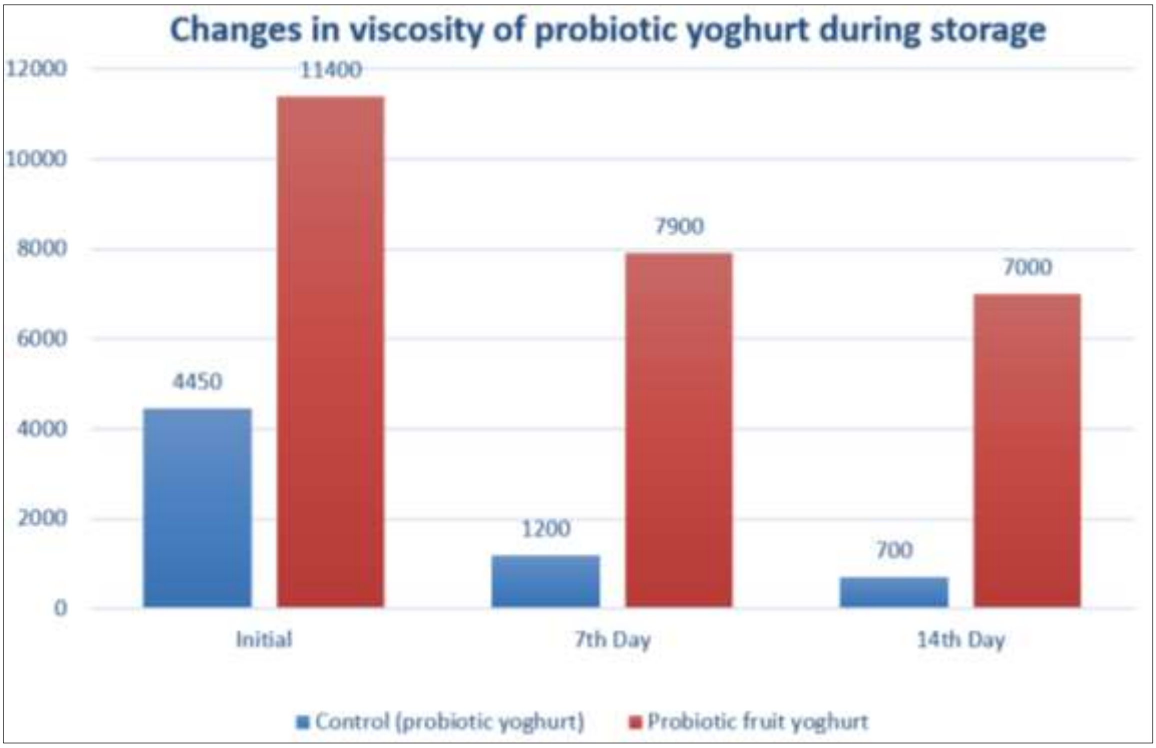
In summary, the table 3 suggests that while T_1 and T_2 are similar to the control, T_3 shows some improvements, and T_4 stands out as the most preferred sample. Further analysis and sensory testing could be conducted to determine the specific factors contributing to these differences and to refine the product for better overall acceptability.

Table 3: Changes in viscosity of probiotic yoghurt during storage

Period	Control (probiotic yoghurt)	Probiotic fruit yoghurt
Initial	4450	11400
7^{th} Day	1200	7900
14^{th} Day	700	7000

Changes in viscosity during storage

The probiotic fruit yogurt, which was prepared using mango, dragon fruit, and apple (initially measuring at 11,400 cP), exhibited a notably high viscosity. In contrast, the control yogurt, initially measured at (4,400 cP), showed a minimum viscosity value (4400 cP). Both probiotic fruit yogurt and the control yogurt experienced a decrease in viscosity during storage. By the end of the 14th day of storage, the probiotic fruit yoghurt variant reached a viscosity of (7,000 cP), significantly higher than the control yogurt, which measured at (700 cP). The probiotic fruit yogurt consistently maintained the maximum viscosity throughout the storage period. These findings align with the research conducted by Lee *et al.* (2007) [8].



Graph 1: Changes in Viscosity during Storage

Table 4: Microbial load during storage

Particulars	Storage period	Control (Probiotic yoghurt)	Probiotic fruit yoghurt
Probiotic count	Initial day	57×10^{12}	42×10^{12}
	14th day	9×10^{8}	21×10^{8}
Yeast & mold *	Initial day	-	-
	14th day	-	-

*Yeast and mold count was nil at 10^3 and 10^5 dilutions.

Initially, the highest probiotic count, measuring 57 x 10^{10}, was recorded in the control sample, with the probiotic fruit yogurt closely behind at 45 x 10^{10}. After 14th day of storage period lowest count of 9 x10^8 was observed in control. In Table 4, it's noted that the maximum probiotic count of 21 x 10^8 was observed in probiotic fruit yogurt after 14 days of storage. The presence of adequate acidity is a crucial factor that can significantly impact the survival and viability of probiotic bacteria in cultured dairy products. In our current study, we observed that the probiotic fruit yogurt consistently maintained a more desirable level of acidity compared to the other samples. This favorable acidity level is likely the reason for the higher count of viable probiotic bacteria in the probiotic fruit yogurt compared to the control samples. Notably, no yeast or mold colonies were observed on the plates throughout the entire 14th day storage period.

Zekai Tarakci (2012) [10] study explored the influence of aloevera on probiotics. Their findings indicated that the overall viable count of probiotics remained at a satisfactory level. Specifically, the population of probiotic strains, such as Lactobacillus acidophilus and *Bifidobacterium bifidum*, consistently exceeded 10^9 colony-forming units per milliliter (cfu/ml) even throughout extended storage periods.

Colour measurement of probiotic yoghurt

The colour analysis of optimized product and control were performed by using colorimeter Hunter Lab, model Colour Quest XE (Reston, Virginia, United States).

Table 5: Estimation of Colour Values by Hunter Lab

Sample	L*	a*	b*
T_0	91.45 ± 0.52^f	1.42 ± 0.12^a	13.43 ± 0.252^b
T_4	60.01 ± 0.89^e	24.64 ± 0.36^d	14.96 ± 0.56^c

*Each value is average of three determinations. Different subscripts in a column represent significant differences (p<0.05).

The change in L value of optimized product was due to inclusion of mixed fruit pulps which decreased the lightness of mixed fruit yoghurt. Furthermore, "a" value indicates degree of redness to greenness of which positive value indicated red color whereas negative value indicates green color (Yeganehzad S, 2007) [9]. "a" value for control product was -1.42 whereas for acceptable product is 24. 64. Positive value of optimized product might be due to addition of fruit pulps. The "b" value indicates degree of yellowness to blueness where a positive number indicate yellow colour and negative number indicates blue colour.

Table 6: Nutritional value of prepared fruit based probiotic yoghurt

Parameter	Energy (Kcal)/100gm
Carbohydrate	19.12 ± 0.17^b
Protein	16.08 ± 0.12^a
Crude Fat	35.55 ± 0.25^c
Energy Value	70.75 ± 1.2^d

*Each value is average of three determinations

Table 6 clearly indicates that the total energy content of the probiotic yogurt amounts to (70.75±1.2) Kcal. This calculation derives from the summation of energy values obtained from total carbohydrates, crude protein, and crude fat.

Conclusions

In conclusion, this study successfully achieved its primary objective of formulating a probiotic yogurt infused with mango, dragon fruit, and apple, incorporating a probiotic culture of *Lactobacillus casei* and *Lactobacillus plantarum*, as well as a yogurt culture, with the added substitution of sugar with jaggery. Comprehensive assessments encompassed nutritional composition, physicochemical characteristics, textural properties, sensory evaluation, and storage dynamics. The resulting probiotic yogurt demonstrated a favorable nutritional profile and distinct color attributes. Standardized preparation methods were established, and the acceptability of various formulations revealed that T_4, containing 79% yogurt and 21% fruit juice, received the highest sensory ratings. Furthermore, the storage investigation demonstrated the stability of the probiotic yogurt over a 14-day period, with significant reductions in total plate counts. Throughout the study, yeast and mold counts remained negligible. These findings contribute valuable insights into the development, acceptability, and storage considerations of probiotic yogurt enriched with diverse fruit flavors.

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